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Antigen cross-presentation: extending recent laboratory findings to therapeutic intervention

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Summary

The initiation of adaptive immune responses requires antigen presentation to lymphocytes. In particular, dendritic cells (DCs) are equipped with specialized machinery that promote effective display of peptide/major histocompatibility complexes (MHC), rendering them the most potent stimulators of naive T lymphocytes. Antigen cross-presentation to CD8$^+$ T cells is an important mechanism for the development of specific cytotoxic T lymphocyte (CTL) responses against tumours and viruses that do not infect antigen-presenting cells. Here, we review recent findings concerning antigen cross-presentation to CD8$^+$ T lymphocytes. Specific subtypes of DCs in the mouse have been defined as being especially endowed for antigen cross-presentation, and a human homologue of these DCs has recently been described. DC vaccination strategies for the prevention and treatment of human diseases have been under investigation in recent years, but have not generally reached satisfying results. We here provide an overview of new findings in antigen cross-presentation research and how they can be used for development of the next generation of human DC vaccines.

Keywords: antigen cross-presentation, CD8 T cell, dendritic cell, HLA, human, MHC, mouse

Antigen cross-presentation to CD8$^+$ T cells, a historical perspective

Dendritic cells (DCs) are key players in initiation and control of adaptive immune responses due to their exquisite ability to present antigenic fragments in the form of peptide/major histocompatibility complexes (MHC) to T cells [1–3]. Endocytosed antigens acquired from the outside environment are generally presented as peptide/class II MHC complexes, while antigens acquired from within the cell are predominantly presented as peptide/class I MHC complexes. This dichotomy raises one complication: how are DCs able to present viral or tumour peptides on class I MHC if these peptides are not endogenously produced? In 1976 a third mechanism was identified, whereby exogenous class I MHC-restricted antigens are captured by DCs, resulting in the induction of CD8$^+$ cytotoxic T lymphocyte (CTL) responses [4]. This process was coined 'antigen cross-priming'. Cross-priming is important in anti-viral and anti-tumour immunity [5,6]. Mouse experiments in which non-haematopoietic cells were virally infected showed a requirement for cross-presentation by haematopoietic cells to elicit virus-specific CTL responses [5,7]. Secondly, antigen cross-presentation is relevant to the induction of central immune tolerance in the thymus [8] and peripheral tolerance in the draining lymph node [9], a process referred to as 'cross-tolerance' [5,8]. In the 1990s, the term 'cross-presentation' was introduced to describe the antigen-presentation process underlying cross-priming and cross-tolerance. Experimental support again came from mouse model-based experiments, such as using the receptor interacting protein-membrane-bound ovalbumin (RIP-mOVA) mice that express a membrane-bound form of ovalbumin on restricted tissues including pancreatic β cells [9]. Analysis of RIP-mOVA thymus and control thymus grafted mice after being injected with OVA-specific CD8$^+$ T cells from OT-I transgenic mice suggested that OVA-specific CD8$^+$ T cells were lost and probably deleted after entry in the peripheral tissues [9]. Also, the inability of DCs to cross-present results in the accumulation of fully functional self-reactive CD8$^+$ T cells that can cause autoimmune disease [10].

DCs, B cells, monocytes and macrophages are classified as prototypic professional antigen-presenting cells (APC) by virtue of their constitutive expression of class II MHC molecules. Professional APCs are critically important for induction of protective CD8$^+$ T cell responses against normal

'self'-antigens [11], tumour antigens [11,12] and viruses [13]. As was already shown elegantly in 1996, the injection of OVA peptide-specific naive $CD8^+$ T cells into non-irradiated RIP-mOVA mice results in selective presence of these T cells in the draining lymph nodes of OVA-expressing tissues (i.e. pancreas and kidney) and not other lymph nodes [11]. These data supported the notion that cross-presentation is a constitutive mechanism, whereby T cells can be primed to antigens that are present in non-lymphoid tissues that are normally not patrolled by circulating naive T cells.

The activation of CTL upon recognition of infection- or tumour-associated peptides encompasses risk to autoimmune T cell reactivity and is therefore under tight control. Under homeostatic as well as inflammatory conditions, tissue-specific DCs and, to a lesser degree, macrophages, execute peripheral tolerance control by their ability to discriminate between cross-presentation and cross-tolerization [14,15]. Also liver sinusoidal endothelial cells are capable of cross-presenting soluble exogenous antigen to $CD8^+$ T cells leading to tolerance [16]. Other cell types are not yet described to have the ability to induce cross-tolerance under those non-inflammatory conditions. During infection, however, more cell types were recently identified as being able to cross-prime foreign peptides and elicit CTL responses. Thus far, B cells [17,18], neutrophils [19,20], basophils [21], mast cells [22] and endothelial cells [23] were also demonstrated to be capable of cross-presentation *in vitro*. Cross-presentation by basophils was even shown to be relevant in an *in vivo* experimental autoimmune encephalitis model [22]. However, the involvement of the other cell types in cross-presentation *in vivo* has not yet been shown, and particularly DCs appear pivotal for antigen cross-presentation in various circumstances as, for example, demonstrated by a lack of CTL responses against cell-associated antigens after depletion of DCs *in vivo* [24]. The efficiency of DCs to cross-present exogenous antigens as peptide/class I MHC *in vivo* was emphasized in a direct comparison study, where cross-presentation showed near equal efficiency as presentation of peptide/class II MHC derived from the same antigen [25].

Specific DC subsets are associated with antigen cross-presentation, and initial descriptions for these subsets are now reported in humans. Various mechanisms that facilitate cross-presentation by DC subsets were especially investigated in the last decade, mainly in mouse-based experiments. Human DC research that involves antigen cross-presentation is lagging behind. This review focuses on the mechanisms and cells that are known to be relevant for induction of effective $CD8^+$ T cell responses to endocytosed antigens.

Mechanisms in DCs that facilitate antigen cross-presentation

The ability of DCs to cross-present antigen to T lymphocytes is not represented uniformly in all DC subsets. Some DC types are more specialized in antigen transport from peripheral tissues to secondary lymphoid tissues, whereas others are non-migratory and are specialized at generation and display of peptide/MHC complexes to naive T cells that reside within lymph nodes. The role of the different subsets of DCs in antigen cross-presentation has been studied extensively in mice. DCs are characterized in the literature as lineage-marker-negative (CD3, 14, 15, 19, 20 and 56) and high expression of MHC class II molecules. Mouse DCs are further marked by expression of the integrin CD11c, and additional delineation can be made using additional cell surface markers [3,26–28].

Although some aspects of the human and mouse DC systems appear to be well conserved, other functions do not relate. In mice, a subset of resident DCs, characterized by high surface expression of CD8α [29], is associated with the ability to cross-present exogenous (such as necrotic) antigens to $CD8^+$ T lymphocytes [30–36]. The transcription factor Batf3 is crucial for the development of these $CD8\alpha^+$ DCs and absence of Batf3 in gene-targeted mice results in defective cross-presentation [37]. In 2010, the human equivalent of the mouse $CD8\alpha^+$ DCs was described. This human DC subset, characterized by the expression of BDCA-3 (CD141) [28], Clec9A [38,39] and the chemokine receptor XCR1 [40] was present in human peripheral blood, tonsils, spleen and bone marrow and represents a major human DC subset expressing Toll-like receptor-3 (TLR-3) [27,41]. Results indicate a dominant role for $CD141^+$ DCs in cross-presentation of necrotic cell-derived antigens to $CD8^+$ T lymphocytes [27], as well as superior cross-presentation of soluble or cell-associated antigen to $CD8^+$ T cells when compared directly with $CD1c^+$ DCs, $CD16^+$ DCs and plasmacytoid DCs cultured from blood extracted from the same donors [40]. The role of this DC subset can now be scrutinized in experimental setups in laboratories across the globe. Although culturing from haematopoietic precursors is possible, the low frequency of naturally occurring $CD141^+$ DCs [1 in 10^4 peripheral blood mononuclear cells (PBMCs)] provides a further challenge before the ultimate goal of translation to clinical application using DCs to alter immune responses can be achieved.

Mechanisms that promote antigen cross-presentation that are inherent to immature DCs include their ability to actively control alkalinization of their phagosomes [42], their low lysosomal proteolysis [43] and expression of protease inhibitors [44], thereby increasing the propensity that exogenous antigens engulfed in the phagosome lumen are cross-presented to $CD8^+$ T cells [43]. However, there are also mechanisms restricted to DC subsets or to DC maturation stages, resulting in variability in cross-presentation efficiency. In some instances, cross-presentation ability by DCs correlates with expression of specific uptake receptors or proteins [45,46]. In addition, the nature of the antigen itself also creates a bias towards presentation via class I or class II MHC molecules [45].

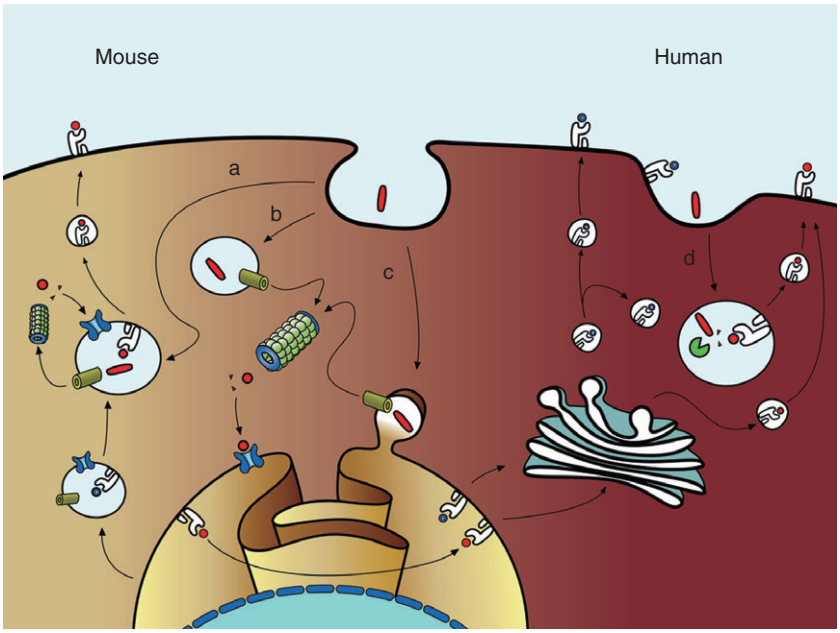

Fig. 1. Proposed pathways of antigen cross-presentation. Yellow area (left side) relates to mechanisms described only in mice so far, whereas the gradient towards the red area (right side) depicts the transient increase in knowledge of antigen cross-presentation mechanisms in human cells. By receptor-mediated endocytosis, the antigen (red rod shape) is engulfed into a phagosome and subsequently processed in a cytosolic proteasome-(a,b,c) or endosomal protease-dependent (d) manner. For proteasome-mediated degradation the antigen is transported across the endosomal membrane into the cytosol by Sec61, accessed by the proteasome via endoplasmic reticulum (ER)–endosome fusion (c) or a delivery vesicle derived from the ER (a,b). After processing by the proteasome, possibly assisted by cytosolic peptidases, peptides either re-enter the endosomal compartment via transporter associated with antigen processing (TAP) where loading on class I major histocompatibility complex (MHC) may occur (a), or the canonical class I MHC presentation pathway in the ER (b,c). After proteolytic processing by endosomal pathway-resident proteases, peptides are loaded onto class I MHC molecules by replacing either exogenous peptide (recycling) or endogenous peptide (classical) loaded on class I MHC complexes (d). : antigen; : exogenous peptide; : endogenous peptide; : Sec61; : TAP; : MHC class I molecules; : proteasome; : proteases.

Once exogenous antigen is internalized by DCs, distinct mechanisms take place by which antigen-derived peptides are cleaved from larger antigen fragments and loaded onto the class I MHC molecules. To allow for display of exogenously acquired antigen in the form of peptide/class I MHC complexes, the antigen undergoes proteolytic processing to create an appropriate-sized fragment. Further restriction to the formation of peptide/class I MHC complexes involves the amino and carboxyl ends of the peptide to harbour charged anchor residues that complement those of the peptide-binding groove of the class I MHC molecule. Because the proteasome is demonstrated to be the main source of peptides in the classical MHC class I pathway, it is not unexpected that proteasome activity is thought to be essential for cross-presentation [13,45,47,48]. However, other reports have shown proteasome-independent processing of the exogenous protein via specific proteases [49,50]. This controversy has led to two different models, the dominant cytosolic pathway and the vacuolar pathway (Fig. 1).

The cytosolic pathway proposes that antigen is transported into the cytosol after internalization where proteasome degradation ensues, prior to transportation to the location of peptide assembly into peptide/class I MHC molecules. Based on the mechanism used by DCs corroborated by the size-restriction of the antigen, internalization of antigens occurs by receptor-mediated endocytosis, pinocytosis (components < estimated 0·5 μm) or phagocytosis (components > estimated 0·5 μm). Upon internalization, antigens are located initially in phagosomes. These phagosomes fuse with early endosomes (characterized by a near-neutral, slightly acidic pH) and later with late endosomes (pH approximately 5·5). Accordingly, ultimate degradation into single amino acids takes place after fusion with acidic lysosomes, a route that is more prevalent in macrophages than in DCs [51]. Degradation within lysosomes occurs by proteases and hydrolases that have their enzymatic optimum close to the acidic pH found in lysosomes (pH 4·8) for antigen degradation [52], as well as degradation of cellular constituents as part of the normal cell homeostasis. The changes that occur to phagosomes in the endocytosis pathway is termed 'phagosome maturation'. Phagosome maturation is important in regulation within the immune system, in the decision process as to whether an immune response is triggered or tolerance is established. The importance of this route is exemplified by changes in cell degradation that have been shown to result in autoimmune disease

[52,53]. For example, in a DNase II$^{-/-}$ interferon (IFN)-IR$^{-/-}$ mouse model, where macrophages were unable to degrade mammalian DNA and started the production of tumour necrosis factor (TNF), activation of synovial cells was observed resulting in chronic polyarthritis symptoms [53].

It can be deduced that the process of protein degradation following phagosome maturation must be tightly regulated. In DCs, the pH in phagosomes is kept near neutral (pH 7·5) for the first few hours after phagocytosis [54]. This is in stark contrast to rapid acidification that is seen in neutrophils and macrophages, where the pH drops to 5 within 30 min after phagocytosis [55]. Acidification of the phagosome, thereby increasing the lysosomal protease activity, has been shown to counteract cross-presentation in mice in a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex 2 (NOX2)-dependent manner [54]. This indicates that DCs have a unique ability to regulate proteolytic activity in phagosomes, therefore controlling the amount of peptide destined for cross-presentation. Rab27a-dependent inhibitory lysosome-related organelles are involved in this pathway. These organelles are recruited continuously to phagosomes and limit acidification and degradation of ingested particles in DCs, thus promoting antigen cross-presentation [56,57]. Being able to interfere with phagosome acidification, thereby executing control of the rate of antigen cross-presentation, could provide new opportunities in increasing the efficacy of CTL targeting in DC vaccination.

The insulin-regulated aminopeptidase (IRAP) was implicated in antigen cross-presentation in peptide cleavage for generation of peptide substrates for class I MHC molecules [58]. IRAP was found in the early endosome of human monocyte-derived DCs and murine bone marrow-derived DCs, where it co-localized with MHC class I molecules and the mannose receptor (MR), but not with endoplasmic reticulum aminopeptidases (ERAPs). IRAP-deficient mice were capable of phagocytosis of antigen as well as presenting endogenously produced peptides, but cross-presented exogenous antigens with 50–70% decreased efficiency compared to wild-type mice [58]. IRAP-dependent cross-presentation requires active proteasome and function of the adenosine triphosphate (ATP)-binding cassette transporter family member TAP (transporter associated with antigen processing), but not lysosomal proteases. Therefore, this route of antigen cross-presentation involves cytosolic antigen degradation that is followed by peptide transport via TAP into IRAP^{+} endosomes. However, IRAP as well as MR appear dispensable for cross-presentation in murine splenic CD8α^{+} DCs but not mouse monocyte-derived DCs induced by inflammation, suggesting a role for these two molecules in inflammatory DCs, but not in steady-state CD8α^{+} DCs [59].

To allow for generation of peptides by the proteasome and cytosolic peptidases, antigen must traverse from the phagosome into the cytosol. Recent reports demonstrate that peptide transfer across the phagosomal membrane occurs via a selective, size-specific, reduction, unfolding (partial proteolysis) and Sec61-dependent process [60–62]. Conversely, TAP transporters appear essential for peptide transport from the cytosol into a class I MHC loading compartment, as TAP knock-out mice are not capable of cross-presenting exogenously acquired viral peptides [7]. Moreover, efficient cross-presentation required TLR-4- and signalling molecule myeloid differentiation factor 88 (MyD88)-dependent relocation of TAP [63], essential for peptide loading of class I MHC, to early endosomes/phagosomes [64]. After processing in the cytosol, the generated peptides are transported via TAP either into the endoplasmic reticulum (ER), thereby entering the canonical class I MHC presentation pathway [13,47], or back into the phagosomal pathway [64–66]. The latter situation is likely to contribute to a rapid cross-presentation, as all necessary components are in a separate class I MHC loading-competent compartment that is distinct from the ER. Peptides generated locally in the phagosomal pathway would not undergo rigorous competition with the large pool of endogenous peptides for association with newly assembled class I MHC complexes, as would occur in the ER. It has indeed been shown that all relevant components of class I MHC loading complexes are present in early phagosomes and that these are functional [47]. How the necessary components are transported from the ER to the phagosomes is not clear. Phagosome–ER fusion was proposed [67], but other groups were unable to confirm these findings [64,68]. The vacuolar pathway is an alternative model that is based on notions of proteasome- and TAP transporter-independent cross-presentation, enabled by proteases that reside in late endosomes and lysosomes [49,69–71]. Most antigen cross-presentation studies performed in human DCs to date focus on this pathway, and less on proteasome/TAP-dependent mechanisms [58,59,71]. As only peptide-bound class I MHC molecules are transported to the plasma membrane, peptide-exchange should be able to occur in the endosomal encountered class I MHC molecules. Earlier *in vitro* experiments have already suggested that peptide-receptive class I MHC molecules can be generated under late endosomal/lysosomal pH conditions [72]. Multiple pathways can co-exist in the same cell type, indicating that these pathways are compartmentalized and require sorting and specific antigen targeting to specialized endosomal compartments [49].

Antigen uptake routes by DCs control antigen cross-presentation efficiency

To allow for the induction of specific adaptive immunity, pathogens or antigenic components that are pathogen-derived must be internalized by DCs for antigen processing and display as peptide/MHC complexes at the DC surface. It is clear that DCs can (cross-)present exogenous antigen without being infected [13]. Antigen can be acquired directly from the surrounding milieu, or can be received by a cross-

Table 1. Receptors involved in targeting antigen to the class I or class II major histocompatibility complex (MHC) antigen presentation pathway in mouse and human dendritic cells [26,38,39,41,75].

	Mouse	Human
Activating Fc receptors		
Fcγ receptors (FcγR)		
Fcγ I (CD64)	MHC I [57,76–80]	MHC I [81]
Fcγ II (CD32)		
Fcγ III (CD16)		
Pathogen recognition receptors (PRRs)		
C-type lectin receptors (CLRs)		
Type I		
Mannose receptor (MR/CD206)	MHC I [45]	MHC II [82]
DEC205 (CD205)	MHC I [23,80]	MHC I [83,84]
Type II		
DC-SIGN (CD209)	n.a.	MHC I [85,86]
Langerin (CD207)	MHC II/ MHC I [80]	n.d.
DCIR (CLEC 4A)	MHC II [46,80]	MHC I [87]
Dectin I	MHC II [88]	MHC I (CLEC 9A) [27]
Dectin II	MHC I [89]	n.d.
LOX-1	MHC I [90]	n.d.
Scavenger receptors (SR)		
SR-A1 and SR-A2	n.d.	n.d.
SR-B1 (CD36)	n.d.	MHC I [91]

The involvement of complement-receptors and Toll-like receptors has not been determined. n.d., not determined; n.a., not applicable.

presenting DC from a distant site through transport by migratory DCs. It has been shown that skin-derived migratory DCs transfer antigen to lymph node-resident DCs for efficient cross-presentation [73]. Secondly, it was shown that tumours secrete exosomes that contain proteins, which can be taken up by DCs. This system can facilitate anti-tumour immunity [12]. Thirdly, DCs use gap junctions to gain peptide antigens from adjacent cells. These peptides can be used thereafter for cross-presentation [74].

To allow for antigen internalization, DCs are equipped with a variety of receptors that can either directly recognize pathogen-associated molecular patterns (PAMPS) or indirectly via plasma complement (activated large proteolytic fragments of complement proteins, C3b, C4b, iC3b and C3d – collectively called C') that binds to complement receptors (CR1/CD35 and CR2/CD21). Immunoglobulins (Ig) present in plasma bind the immunoglobulin receptors (activating receptors FcRI, IIA and III and the inhibitory FcRIIB). Both complement fragments and Ig are soluble receptors present in plasma that bind structures on pathogen surfaces to facilitate pathogen opsonization, internalization and destruction. Secondly, small proteolytic complement protein fragments (i.e. C3a, C4a, C5a) act as chemoattractants to recruit and activate new phagocytes. CRs and FcRs allow for internalization after binding C'- or Ig-opsonized antigens. Antigen opsonization with Ig rather than C' facilitates antigen cross-presentation [57], thus supporting a role for Ig rather than C' in tailoring appropriate antigen-specific adaptive immune responses.

DCs use multiple additional membrane-expressed receptors for the internalization of antigens. The presence and dominance of these receptors differs between DC subpopulations within species and between DC subpopulations, as compared between mouse and human [26]. Targeting specific receptors can drive the immune response either towards class II MHC-restricted $CD4^+$ T helper cell responses or to class I MHC-restricted $CD8^+$ cytotoxic T cell responses via cross-presentation, and can therefore be an effective method for inducing anti-viral or anti-tumour CTL responses [46]. In both mice and humans, the presence of many different uptake receptors has been shown (Table 1).

Uptake via distinct endocytic receptors controls the efficiency of cross-presentation of peptide/class I MHC complexes to $CD8^+$ T cells. The effects of individual uptake receptors on antigen targeting to the class I or class II MHC presentation route seem to be roughly conserved between mice and humans, but opposing effects of some receptors related to endosomal targeting and processing of antigens have been found. In mice, antigen cross-presentation is promoted when antigen uptake occurs via MR [45], DEC205 [80,83,88,92,93], dectin-2 [89], DNGR-1 [94], FcγR [57,76–80] and LOX-1 [90]. Also in human cells, antigen cross-presentation is promoted upon antigen uptake via DEC-205 [83,84], DC-SIGN [85,86] and FcγR [81] *in vitro* as well as *in vivo* MR targeting in a humanized mouse model [95]. However, in contrast to the results in mice, MR-mediated antigen uptake induced $CD4^+$ T cell responses by human DCs [82]. Antigen targeting to dectin-1 [88], DCIR-2 [46,80] and CD40 [96] induce $CD4^+$ T cell responses in the mouse. Langerin (CD207)-targeted uptake induces both $CD4^+$ and $CD8^+$ T cell responses [80]. In humans, antigen cross-presentation is favoured by uptake via DCIR

[87]. These examples underscore that knowledge on receptor-mediated cross-presentation in mice cannot always be translated immediately to the human system. Because enhancing cross-presentation can be an effective means to improve CTL responses in diverse DC vaccination programmes, more research about receptor targeting in the human system is needed.

Antigen uptake routes in DCs can be decisive in induction of immunity or tolerance

Cytotoxic $CD8^+$ T cells directed to virus-infected cells are considered crucial for efficient anti-viral responses. In parallel, the elicitation of tumour-directed CTLs is considered crucial for effective anti-tumour responses to occur. Considering tumour-associated antigens, dead tumour cells are a major antigen source for APCs [91]. For example, Asano *et al*. showed that dead tumour cells traffic via the lymph vessels to the tumour-draining lymph node where dead tumour cell-associated antigens are internalized by APCs and cross-presented to $CD8^+$ T cells [97]. In cancer therapy, many investigators have taken advantage of the immunogenicity of tumour-associated antigens for tumour vaccination, either by direct injection of dead tumour cells [98] or using DCs loaded with dead tumour cells [99].

For pathogen-associated antigens, a large pool of antigens is also available in cells that are dead or dying as a consequence of the pathogen infection, forming a rich source of antigens for loading into the cross-presentation pathway. It is demonstrated that cells dying from infection are engulfed by APCs for $CD8^+$ T cell activation by cross-presentation. For example, virally infected dying cells such as influenza A [91], Epstein–Barr virus [100] and canarypox virus [101] or bacterially infected dying cells from *Salmonella typhimurium* [102] induce $CD8^+$ T cell responses. It is clear from these examples that providing antigen in the form of dead cells can be a powerful tool to favour cross-presentation of the antigen.

However, not all dead cells are immunogenic and induce cross-presentation. In humans, it is estimated that under homeostatic conditions approximately 1 million cells turn over each second, which does not generally result in autoreactivity [103]. However, deficiencies in the clearance of these dead cells can results in autoimmune disorders (i.e. systemic lupus erythematosus in individuals lacking early components of the complement cascades), indicating its role in maintenance of self-tolerance. Clearly, this example shows that the immune system is able to process dead cells in a tolerogenic or immunogenic manner, depending on several factors. As reviewed by Green *et al*., these factors are related to the type of cell death, the cell death pathway, how the dead cells are engulfed, the engulfing cell, where the engulfment takes place and which cells of the immune system eventually encounter the antigens presented along with the dead cells [104]. Clarification (and possibly modulation) of these processes should provide a venue for development of efficient cross-presentation routes that can be exploited in DC vaccination strategies. Several such attempts are exemplified in experiments on heat shock protein (HSP)-associated antigen uptake. HSPs are intracellular chaperone molecules that associate readily with neighbouring proteins, such as with antigen inside tumour cells. Injection with HSP, e.g. HSP 70, HSP 90 and glycoprotein (gp)96 induces CTL responses against the cells from which the HSPs were isolated (reviewed in [105]). While HSPs may not be essential for antigen cross-presentation, they have been shown to promote antigen cross-presentation using *in vitro* assays employing multiple cell lines as well as primary mouse and human immune cells [105–109]. The myeloid differentiation factor 88 (MyD88) is essential in the developmental maturation of DCs that allows them to prime $CD8^+$ T cells through cross-presentation after uptake of HSP-coupled antigen [63].

Clinical experience with anti-tumour and anti-viral DC-vaccines

In recent years, multiple insights were obtained in mechanisms that underlie cross-presentation in mouse as well as human cells. The primary cross-presenting $CD8\alpha^+$ DC in mice was identified, and groups around the world are currently investigating what seems to be a human homologue, the human $CD141^+$ DC. Distinct pathways are shown to be present in the murine system, and more knowledge is being increasingly gathered about the cross-presentation pathways in the human system. These mechanisms are not always conserved between species, which alerts us that knowledge gathered in mouse systems cannot be extrapolated to the human system without risk.

While our knowledge in antigen presentation biology increases, the potential benefit of its function is being explored in patients. In the last 15 years, at least 50 Phase I and Phase II trials in humans were performed using DC vaccination as anti-tumour [45,110–118] or anti-viral treatment [119,120] related to allogeneic stem cell transplantation (SCT) in both adult and paediatric settings (references [113,115–118] and [110–112,114,120], respectively). The main focus of these Phase I/II studies was safety, and none reported serious direct side effects. Although the studies were not set up for evaluation of effectiveness of induction of CTL responses, disease regression/prevention and immune responses were measured. In general, anti-tumour responses were minimal [110,112,114,117], as reviewed by Rosenberg *et al*. [121]. One recent study performed in 10 AML patients after at least one anti-leukaemic chemotherapeutic regimen, but not end-stage disease, showed more promising results. In this study, vaccination with DCs loaded with mRNA encoding Wilms' tumour 1 (WT1) protein induced complete remission in three of 10 patients and temporary remission in two additional patients. Moreover, an increased frequency of

WT1-specific CD8+ T cells was found in two of five tested HLA-A0201+ patients, correlating significantly with long-term response [118].

There are more examples of DC vaccination studies in which the induction of T cell responses was measured. For example, in studies in stage IV melanoma patients, peptide-pulsed CD34+ progenitor cell-derived DCs induced both CD4+ and CD8+ T cell responses [115,116]. Also, in patients suffering from breast cancer, DCs loaded exogenously with peptides derived from the human epidermal growth factor 2 (HER-2)/neu proto-oncogene and the epithelial mucin MUC1 lead to an induction of peptide-specific CTLs and decrease in serum amount of tumour markers [113]. DC vaccination in 35 non-Hodgkin B cell lymphoma patients targeting tumour-specific immunoglobulin resulted in a tumour regression rate of 31·6% [122]. In studies aimed at the induction of anti-viral immunity after allogeneic SCT, targeted mainly at human cytomegalovirus (HCMV), vaccination with pp65-pulsed DCs induced a sustained antigen-specific CD4+ T cell response; CTL responses were not assessed [120]. HCMV peptide-loaded DCs induced HCMV-specific CTL responses in five of 24 SCT patients at risk for HCMV after allogeneic SCT [119].

As described, the results from anti-tumour and anti-viral clinical trials show a modest immunological response, which may not yet result in an increase in patient survival. As most of these studies were designed as Phase I and Phase II safety studies the included patient groups tend to be late in disease progression (with a relatively high residual tumour load or already with virus-associated disease). Patients with a substantially lower tumour load and absent viral disease may be more likely to show benefit from induced specific anti-tumour or anti-viral activity, as also suggested by recent results [118]. Conversely, the potency of immunological responses was far from optimal, and provides opportunities for improvement. New information on human DC subsets and education of DCs allow for the optimization and improvement of current DC vaccination strategies. Distinct DC subsets offer unique possibilities in DC vaccination strategies [123]. In addition, priming DCs in a specific way determines the immunological outcome, which can be accomplished by inclusion in vaccine formulations of ligands to TLRs [124] and non-obese diabetic-like receptors (NLRs) [125] and may work through modulation of the DCs migratory and T cell stimulatory capacity. Secondly, in reported studies thus far, the elicitation or reactivation of CD4+ T cell responses can often be observed, and even humoral anti-tumour responses can be measured. Class I MHC-restricted CD8+ T cell responses are the focus in only a minority of papers, while it is CTLs that are key players in anti-tumour and anti-viral immunity. Therefore, the induction of potent antigen cross-presentation should be especially explored in current human immunology studies. Clarification of the mechanisms that increase the amount of peptide/class I MHC complexes is pivotal for the development of next-generation DC-based anti-tumour and anti-viral intervention strategies.

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Disclosure

The authors declare no conflicts of interest.

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